OSMOREGULATION IN THREE MOLLUSCS: *ACANTHOCHITONA DISCREPANS* (BROWN), *GLYCYMERIS GLYCYMERIS* (L.) AND *MYTILUS EDULIS* (L.).

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Molluscs certainly constitute one of the most representative phyla of the littoral area, inasmuch as they have often been chosen as landmarks in the definition of the shore levels. In these biotopes, molluscs withstand frequent and rapid changes in the osmotic pressure of their environmental medium. The euryhalinity of aquatic molluscs has been the subject of many investigations (for review see Robertson, 1964; Schoffeniels and Gilles, 1970a) and it is classically assumed that, in these species, this is the cell which has to cope with the osmotic stress. Marine molluscs are indeed considered as poecilosmotic animals having no power of anisomotic extracellular regulation. However, if the blood of the periwinkle *Littorina* is isosmotic with the medium in salinities down to 17‰, it becomes hyperosmotic in more diluted media, the mean blood $\triangle$ being 1.06° C against 0.48° C for the medium (Todd, 1964). In the same way, a mean blood $\triangle$ of 0.74° can be recorded for specimens of the bivalve *Scrobicularia plana* acclimated to a medium of 0.59° C (Freeman and Rigler, 1957) and the salinity inside the mantle cavity of *Mytilus* can be 24‰ when the salinity outside is only 7‰ (Milne, 1940). Since the hyperosmotic state observed in marine gastropods submitted to an osmotic stress is known to be due to the ability of these animals to close tightly their internal cavity, one wonders, therefore, if the hyperosmotic state reported in *Mytilus* or *Scrobicularia* is not due, at least partially, to such a "shell-closing" mechanism.

This study deals, therefore, with the incidence of the "shell-closing" mechanism on the ability of some bivalve molluscs to withstand an osmotic stress. Moreover, since amino acids play a part in the regulation of the cellular osmotic pressure in molluscs (for review, see Schoffeniels and Gilles, 1970a), the changes in the intracellular amino-acid pool during acclimation of the studied species to various media have also been considered.

MATERIALS AND METHODS

The studied species—*Glycymeris glycymeris* (L.), *Mytilus edulis* (L.) and *Acanthochitona discrepans* (Brown)—were brought from the Roscoff Marine Laboratory (France) and allowed to recover in the laboratory for two weeks

before use. All the experiments have been carried out at 17° C. In a first series of experiments, lots of 75 to 100 animals of each species were acclimated from sea water to different media (50%–25% sea water or fresh water) either directly or by gradual steps. During the time course of the acclimation, samples of the body fluids have been taken for osmotic pressure and ionic concentration determinations. Perivisceral fluid samples have been taken in the perivisceral cavity
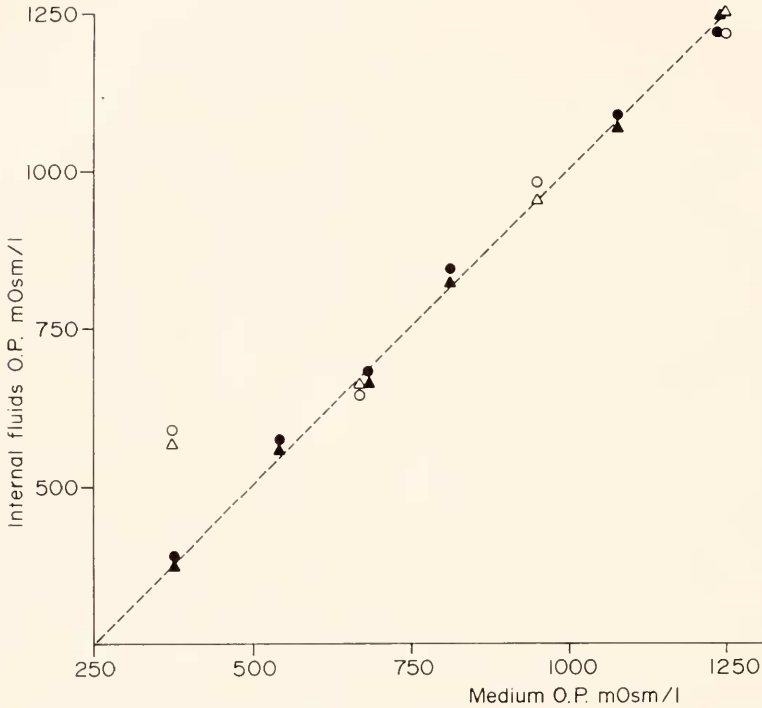

FIGURE 1. Changes in the osmotic pressure of the blood (○) and of the perivisceral fluid (△) of *Glycymeris glycymeris* during rapid (open data points, ○, △) or slow (closed data points, ●, ▲) acclimatization to diluted media. The osmotic pressure is given in milliosmoles/l; dashed line: isosmoticity line. Points are the average of 5 determinations, each one being performed on a sample obtained by pooling the blood or the perivisceral fluid of 4 to 10 animals. The standard deviation never exceeds 4.8%.

while maintaining the valves ajar. Blood samples were collected by direct puncture in the pericardiac cavity. Osmotic pressure measurements of these fluids have been achieved with a mechrolab osmometer. Measurements of Na^+ and K^+ concentrations have been run with a flame photometer and Cl^- concentration has been estimated by potentiometric titration.

In another series of experiments, the molluscs were maintained either in sea water or in 50% sea water for threee weeks. Samples of adductor muscle or foot muscle were then taken for amino-acid and water content determinations. The concentration of the free intracellular amino acids was determined with a

"Technicon autoanalyzer" following the method previously described (Gilles and Schoffeniels, 1968). The water content was estimated by the fresh weight-dry weight technique.

(A) Body fluids osmotic and ionic concentrations

During rapid acclimatization of *Glycymeris glycymeris* to media of various salinities, the blood and the perivisceral fluid of the bivalve remain in osmotic equilibrium with the environmental medium down to salinities of 50% sea water.
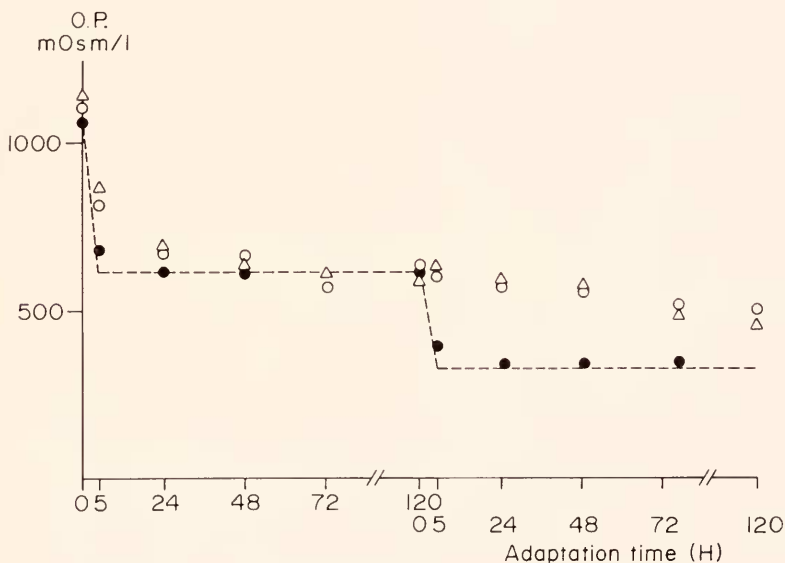

FIGURE 2. Changes in the osmotic pressure of the blood (○) and of the perivisceral fluid (△) of *Glycymeris glycymeris* (open data points, ○, △) or *Acanthochitona discrepans* (closed data point, ●) during the time course of acclimatization to diluted media. The osmotic pressure is given in milliosmoles/1; dashed line: external medium osmotic pressure. Points are the average of 5 determinations, each one being performed on a sample obtained by pooling the blood or the perivisceral fluid of 4 to 10 animals. The standard deviation never exceeds 4.8%.

However, a hyperosmotic state is observed in both blood and perivisceral fluid when *Glycymeris* is acclimated to 25% sea water (Fig. 1). In these experiments, the animals remained two days at each dilution step. If the animals are maintained one week in each dilution, the hyperosmotic state observed in low salinities is no longer recorded and both blood and perivisceral fluid are in osmotic equilibrium with the external medium (Fig. 1). We have, therefore, undertaken experiments attempting to show the effect of the time of acclimatization to various media on the ionic composition of the blood and the perivisceral fluid of various molluscs. The results given in Figures 2 to 5 show the modification of the

osmotic pressure as well as of the Na, K and Cl concentration of the blood and
the perivisceral fluid during the time course of acclimation of *Glycymeris* and
Acanthochitona to 50 and 25% sea water. At each dilution tested, the Na and
Cl concentrations as well as the osmotic pressure of the blood of *Glycymeris*
follow the one of the perivisceral fluid. The osmotic pressure and the Na and
Cl concentrations of this last fluid stay, however, higher than the one of the
external medium for at least 72 hours (Figs. 2, 3 and 5). The perivisceral fluid
reaches an isosmotic equilibrium with the environmental medium only after 120
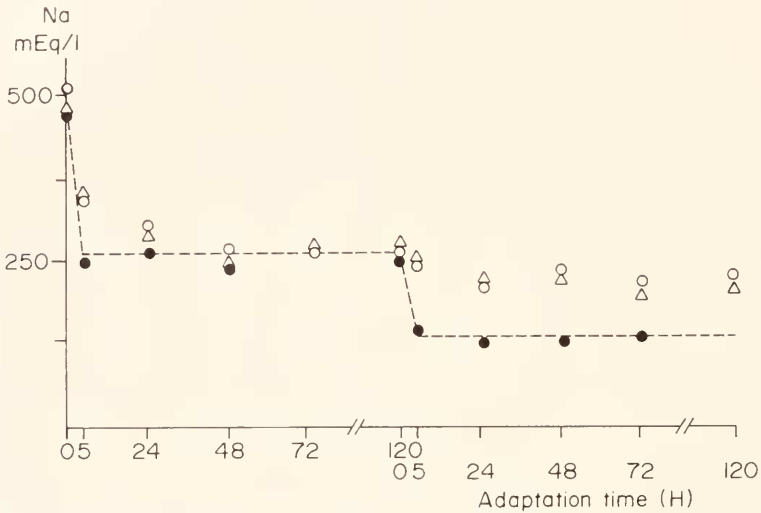hours. The changes in the perivisceral fluid K concentration follows the same

FIGURE 3. Changes in the Na concentration of the blood (O) and of the perivisceral
fluid (△) of *Glycymeris glycymeris* (open data points, O, △) or *Acanthochitona discrepans*
(closed data point, ●) during the time course of acclimatization to diluted media. The Na
concentration is given in mEq/l; dashed line: Na concentration of the external medium.
Points are the average of 5 determinations, each one being performed on a sample obtained
by pooling the blood or the perivisceral fluid of 4 to 10 animals. The standard deviation
never exceeds 4.8%.

pattern. However, it should be noted that the blood K concentration is regulated
at the value it has in the blood of sea-water *Glycymeris* (Fig. 4).

The results obtained with the euryhaline intertidal bivalve *Mytilus edulis*
are similar to the ones described above for *Glycymeris*. In the case of *Mytilus*,
indeed, the blood osmotic pressure remains similar to that measured in the peri-
visceral fluid. At high dilutions of the external medium (25% sea water and
fresh water), the perivisceral fluid remains hyperosmotic to the environmental
medium at least for 96 hours (Fig. 6). This is at variance with what happens
in *Acanthochitona discrepans* where the blood always stays in isotonic equilibrium
with the external medium (Figs. 2, 3 and 5). However, as it is the case for
Glycymeris, the blood K concentration is regulated at the value it has in the blood
of sea water acclimated animals (Fig. 4).

(B) Intracellular free amino-acid pool

As shown in Table I, the concentration of the amino acids is higher in the adductor muscle of *Mytilus edulis* than in the adductor muscle of *Glycymeris glycymeris* or in the foot muscle of *Acanthochitona discrepans*. Moreover, the amino-acid content decreases during the acclimation of these species from sea water to 50% sea water. This decrease mainly affects alanine, aspartic acid, glutamic acid, glycine, proline, serine and the amine, taurine. During hypoosmotic stress, the water content of the muscle increases by 7.8 to 10.6%. However, this increase cannot account for the reduction in the amino-acid and taurine content observed during the acclimatization to the diluted medium (Table I).
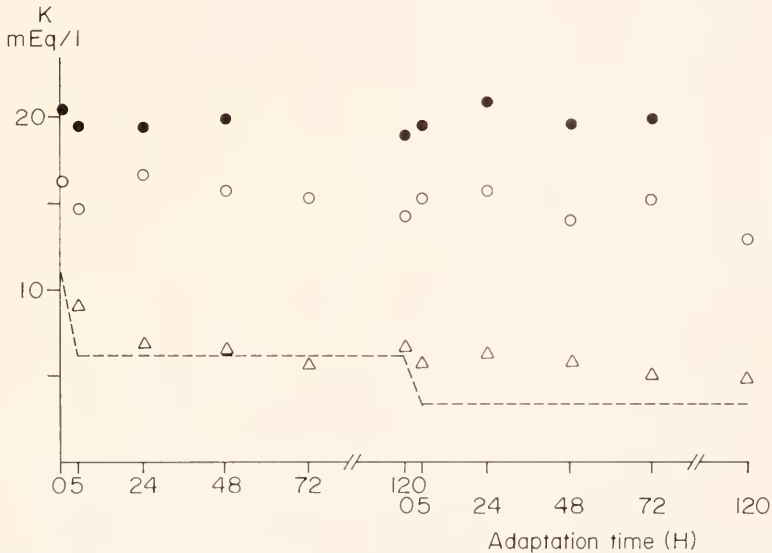

Figure 4. Changes in K concentration in the blood (○) and in the perivisceral fluid (△) of *Glycymeris glycymeris* (open data points, ○, △) or *Acanthochitona discrepans* (closed data point, ●) during the time course of acclimatization to diluted media. The K concentration is given in mEq/l; dashed line: K concentration of the external medium. Points are the average of 5 determinations, each one being performed on a sample obtained by pooling the blood or the perivisceral fluid of 4 to 10 animals. The standard deviation never exceeds 4.8%.

Discussion

A hyperosmotic state can be observed in the body fluids of the bivalve mollusc *Glycymeris glycymeris* when subjected to a rapid acclimatization to 25% sea water. In these experiments, the animals remain for two days at each dilution step. If the animals are now maintained for one week in each medium, the hyperosmotic state observed in low salinities during rapid acclimatization is no more recorded and both the perivisceral fluid and the blood remain in osmotic equilibrium with the external medium. These results suggest that the hyperosmotic state observed during the rapid acclimatization of specimens of *Glycymeris* to

diluted media is not due to a mechanism of anisosmotic regulation but rather to a mechanism attempting to isolate the animal from the external medium. Moreover, this mechanism appears to be effective only during a short period of time. We have, therefore, undertaken experiments in order to show the effect of the time of acclimatization to a diluted medium on the ionic composition of the body fluids of various molluscs. For these experiments, we have chosen two bivalves: *Glycymeris glycymeris* and *Mytilus edulis*. These species can isolate themselves
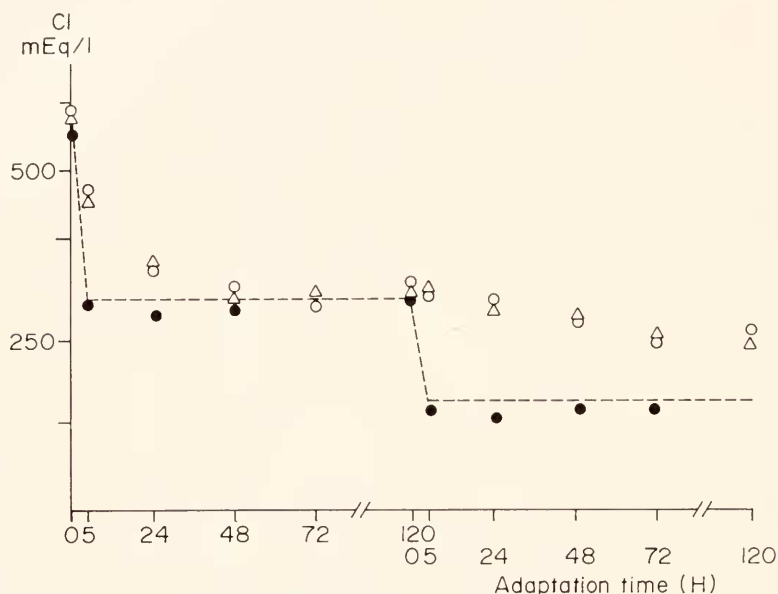

FIGURE 5. Changes in the Cl concentration of the blood (○) and of the perivisceral fluid (△) of *Glycymeris glycymeris* (open data points, ○, △) or *Acanthochitona discrepans* (closed data point, ●) during the time course of the acclimatization to diluted media. The Cl concentration is given in mEq/1; dashed line: Cl concentration of the external medium. Points are the average of 5 determinations, each one being performed on a sample obtained by pooling the blood or the perivisceral fluid of 4 to 10 animals. The standard deviation never exceeds 4.8%.

from the environmental medium by closing tightly their valves. We have also used the Polyplacophora *Acanthochitona discrepans* which does not have such a "shell-closing" mechanism.

When the bivalves *Glycymeris* or *Mytilus* are submitted to hypoosmotic stress, the blood stays hyperosmotic to the external medium for at least 96 hours after the beginning of the acclimatization (Figs. 2 and 6). It has, however, to be noted that the blood, even when hyperosmotic to the external medium always remains isosmotic to the perivisceral fluid. On the other hand, the transitory hyperosmotic state observed in the blood of the bivalves when acclimated to a dilute medium is not recorded during the acclimatization of the Polyplacophora *Acanthochitona*. In this species indeed, which does not have a perivisceral fluid, the blood always remains in osmotic equilibrium with the surrounding medium.

The isosmotic equilibrium is reached immediately after the beginning of the accli-
matization. It can, therefore, be concluded that the three species studied do not
have any anisosmotic regulation mechanism since the blood always remains in
osmotic equilibrium with its surrounding fluid. Moreover, the transitory hyperos-
motic state observed in the blood of the two bivalves studied appears to be due to
the fact that the perivisceral fluid is transitorily maintained hyperosmotic to the
external medium. The same kind of results has been obtained with the gastropods
Littorina saxatilis (Avens and Sleigh, 1965), *Siphonaria pectinata* (McAlister
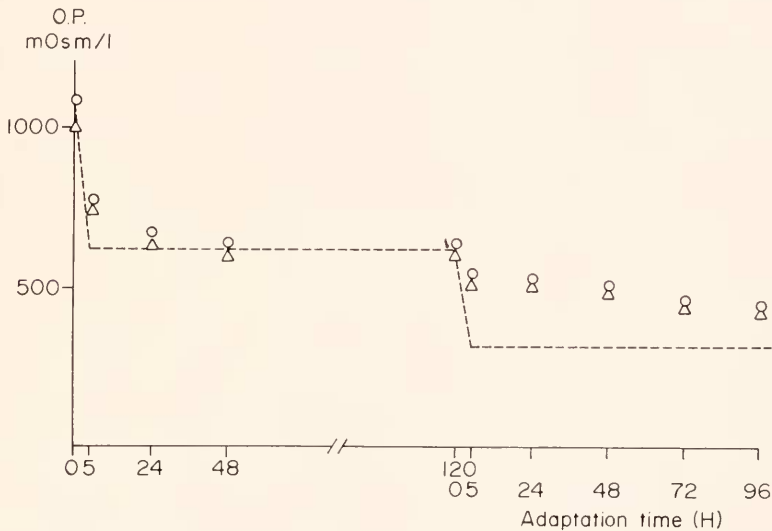

FIGURE 6. Changes in the osmotic pressure of the blood (○) and of the perivisceral
fluid (△) of *Mytilus edulis* during the time course of acclimatization to diluted media. The
osmotic pressure is given in milliosmoles/1; dashed line: external medium osmotic pressure.
Points are the average of 5 determinations, each one being performed on a sample obtained
by pooling the blood or the perivisceral fluid of 4 to 10 animals. The standard deviation
never exceeds 4.8%.

and Fisher, 1968) and *Littorina littorea, Purpura lapillus* and *Patella vulgata*
(Hoyaux and Jeuniaux, in preparation). These last authors, moreover, demon-
strate that the disequilibrium between the perivisceral fluid and the surrounding
medium disappears if the operculum of *Littorina* is removed.

It appears, thus, that some molluscs may be helped in withstanding a sudden
osmotic stress by isolating themselves from the external medium. The temporary
hyperosmotic state so obtained, which has been sometimes interpreted as the effect
of an osmotic regulation in diluted media (see Todd, 1964) is achieved by various
processes. Bivalves such as *Mytilus* or *Glycymeris* close their valves tightly,
gastropods with an operculum such as *Littorina* or *Purpura* retract themselves
strongly in their shell and *Patella* or *Siphonaria* adhere firmly to the rock. Such
a behavior appears to be of considerable importance in the survival of these
animals under temporary adverse conditions as is often the case in the intertidal
or estuarine environment. This "shell-closing" mechanism can only help the animal

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TABLE I

Amino-acid concentration in the muscle of Glycymeris glycymeris, Mytilus edulis
and Acanthochitona discrepans *adapted to sea water or to 50% sea water.*
The concentration of the various compounds is given in $\mu M/g$
wet weight tissue, tr: traces;—: not determined

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Amino acids	Glycymeris glycymeris adductor muscle		Acanthochitona discrepans foot muscle		Mytilus edulis adductor muscle	
	Sea water	50% Sea water	Sea water	50% Sea water	Sea water	50% Sea water
Alanine	7.60	7.10	1.80	1.70	28.07	8.77
Arginine	6.70	6.80	3.40	3.10	—	—
Aspartic acid	9.60	9.70	1.50	1.00	11.25	2.01
Glutamic acid	5.40	4.20	4.00	4.70	5.00	1.03
Glycine	2.10	1.30	0.60	0.70	132.69	58.92
Histidine	0.70	0.20	tr.	tr.	0.58	0.72
Isoleucine	0.20	0.10	tr.	0.10	0.90	0.35
Leucine	0.20	0.20	tr.	0.20	1.57	0.53
Lysine	0.60	0.70	0.30	0.40	1.09	0.72
Phenylalanine	0.80	0.05	tr.	0.10	0.60	0.40
Proline	tr.	tr.	0.40	0.40	45.65	10.95
Serine	3.40	1.40	0.70	0.90	2.86	3.17
Threonine	—	—	0.40	0.40	3.07	1.21
Tyrosine	tr.	tr.	0.10	0.10	0.90	0.58
Valine	—	—	0.20	0.20	2.24	0.75
Taurine	47.10	40.30	17.83	15.24	20.50	9.21
Water content	73.40	81.20	75.10	82.60	71.62	77.26
Osmotic pressure due to amino acids*	56.46	43.45	19.83	18.83	366.49	129.46
Osmotic pressure due to taurine*	71.30	55.14	26.35	20.48	31.77	13.23
Osmotic pressure of the external medium**	1180	573	1180	573	1180	573

* The osmotic pressure due to the various compounds is given in milliosmoles/kg tissue water (Δ of a molar solution of amino acids is $-1.86°$ C—Prosser, Bishop, Brown, Kahn and Wulff, 1950).

** The osmotic pressure of the external medium is given in milliosmoles/l.

to wait for better conditions during a relatively short period of time and, at any rate, it cannot contribute to the osmotic regulation observed in molluscs during modification of the salinity of their environmental medium.

Although the blood of the species we have studied always remains in osmotic equilibrium with its surrounding fluid, the results we have obtained demonstrate that these species are capable of ionic regulation. Indeed, if Na and Cl concentrations are generally close to those of the surrounding fluid, the blood K concentration is regulated at the value it has in the blood of sea water animals (Fig. 4). This regulation of the blood K level has also been observed by Hoyaux and Jeuniaux (in preparation) when working with *Patella, Littorina* and *Purpura*. It is, however, impossible so far to determine if the maintenance of the potassium concentration at a given level is important or not in the osmoregulation process. Further studies are needed to bring some light on this problem.

The results discussed so far show that the studied molluscs have practically no anisosmotic regulation power. One can, therefore, consider that during the acclimatization of these species to a dilute medium, it is the cells which will have

to cope with the osmotic stress. It has been shown by Florkin and co-workers that in molluscs, as well as in other euryhaline invertebrates, aminoacids play a part in the cellular osmotic regulation process. As a matter of fact, the concentration of the free amino acids is higher in the tissue of an euryhaline invertebrate when it is acclimated to sea water than when it is acclimated to a diluted medium (for review see Florkin, 1962, 1966; Florkin and Schoffeniels, 1969; Schoffeniels and Gilles, 1970a, 1970b). The results listed in Table 1 show that in the species we have used, the concentration of the amino acids and taurine is higher in the muscle of sea water acclimated animals than in the same tissue taken from animals acclimated to 50% sea water. The data we have obtained with *Mytilus* are in agreement with those given previously by Bricteux-Gregoire, Duchateau-Bosson, Jeuniaux and Florkin, (1964). On the other hand, the changes observed in the amino-acid concentration are greater than those which could be expected from the increase in the tissue water content. One should, therefore, consider these modifications as being the result of an active regulation process.

When comparing the changes in the concentration of individual amino acids in the three species studied, it is apparent that the changes in the amino-acid concentration do not follow a single general pattern. As a matter of fact, alanine acts as an osmotic effector only in *Mytilus* while the concentration of glycine decreases during the acclimatization to 50% sea water in both *Glycymeris* and *Mytilus*. Moreover, the part played by the amino acids in the isosmotic intracellular regulation of *Acanthochitona* and *Glycymeris* appears to be restricted. Indeed, in the sea water acclimated animals, the total amino-acid concentration accounts for about 2% of the total osmotic pressure in the muscle of *Acanthochitona* and 5% in *Glycymeris*. A greater osmoregulatory role appears to be played by taurine. This compound alone accounts indeed for about 2% of the total osmotic pressure in the muscle of *Acanthochitona,* 6% in *Glycymeris* and 3% in *Mytilus*. The important part played by taurine as a cellular osmotic effector appears to be of general occurrence in the phylum Mollusca (Awapara, 1962; Lange, 1963; Schoffeniels and Gilles, 1970a).

If amino acids and taurine can be considered as intracellular osmotic effectors, playing a part in the cellular osmotic regulation, nothing is known about the mechanisms implicated in the control of their concentration. Evidence has been given that, in Crustacea, the regulation of the intracellular amino-acid concentration, is due at least partly, to a mechanism involving modification of the cellular membrane permeability to amino acids (Gilles and Schoffeniels, 1969; Vincent-Marique and Gilles, 1970a, 1970b; Gerard and Gilles, 1971; Bouguegniau and Gilles, in preparation) and to a mechanism controlling, at the enzymatic level, the metabolism of the amino acids (Gilles, 1969; Schoffeniels and Gilles, 1970b). That modifications of the cellular membrane permeability are involved in the regulation of the amino-acid concentration in molluscs is indicated by the fact that upon exposure of isolated ventricles of *Modiolus* to a hypoosmotic stress, there is an increase in the efflux of the ninhydrin positive substances from the tissue into the saline (Pierce and Greenberg, 1970). On the other hand, the fact that in many euryhaline molluscs, osmotic acclimatization is paralleled by a modification of the oxygen consumption (Hiscock, 1953; Bielawski, 1961; Negus, 1968) and of the ammonia excretion (Emerson, 1969) can be interpreted as an indication of

an increased degradation of the amino acids (Emerson, 1969). This is in agreement with the hypothesis according to which the regulation of the intracellular amino-acid pool may partly depend on a mechanism controlling the relative rate of anabolism and catabolism of these compounds (Gilles and Schoffeniels, 1964; Schoeffeniels, 1968; Gilles, 1969; Gilles and Schoffeniels, 1969). From the results obtained so far in this field with molluscan species, it is, however, hazardous to draw definitive conclusions. A study of the permeability to amino acids as well as of the amino-acid metabolism in tissues of euryhaline molluscs subjected to an osmotic stress is still lacking and further experimental results are needed to bring more evidence in favor of the interpretation given above.

Summary

The molluscs *Glycymeris glycymeris, Mytilus edulis* and *Acanthochitona discrepans* can be acclimated from sea water to salinities down to 25% sea water. During this acclimatization, these molluscs do not show any extracellular anisosmotic regulatory power except for potassium, the blood level of which is regulated at the concentration it has in the blood of sea water aclimated animals.

During rapid acclimatization to diluted media, a transistory hyperosmotic state can be recorded in both blood and perivisceral fluid of the two bivalves species (*Glycymeris glycymeris* and *Mytilus edulis*) but not in the blood of the polyplacophora *Acanthochitona discrepans*. This hyperosmotic state, which can last for about 96 hours in both bivalves when placed suddenly in diluted media, is due to the ability of those molluscs to isolate themselves from the external medium by closing their valves tightly. This "shell-closing" mechanism may help the animals in withstanding a sudden osmotic stress but it cannot contribute to the osmotic regulation observed in these species.

In the studied species, it is the cell which has to cope with the osmotic stress and amino acids play a part in the cellular osmoregulation process. Taurine also appears as an important osmotic effector. Our results show that the concentration of the intracellular free amino acids and of taurine is higher in the muscle of sea water acclimated animals than in the same tissue taken from animals adapted to 50% sea water. The observed changes are greater than those which could be expected from the increase in the tissue water content recorded during the hypoosmotic stress.

The possible mechanisms implicated in the regulation of the amino acids and taurine concentration during the acclimatization of molluscs to diluted media are discussed.

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